FLORAL INDUCTION GENE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] Not applicable.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with United States government support awarded by NSF Grant No. 9318481. The United States has certain rights in this invention.

BACKGROUND OF THE INVENTION

[0003] The present invention relates to the control of flowering time in plants using genetic engineering. Specifically, this invention relates to the control of flowering time in plants by manipulation of the activity of the FPA gene.

[0004] In growing plantlets, the transition from vegetative growth to flowering is the major developmental switch in the plant life cycle. The timing of this transition to a flowering state is critical for the plant's reproductive success. Accordingly, most plant species have evolved systems to precisely regulate flowering time. These systems monitor both environmental cues and the developmental state of the plant.

[0005] Photoperiod and temperature are two environmental cues commonly monitored by plants. In plants responsive to photoperiod cues so examined, flowering is promoted by flowering signals which are translocated from leaves to meristems as leaves detect day length changes (Zeevaart, Light and the Flowering Process, 137-142 (Eds., D. Vince-Prue, B. Thomas and K. E. Cockshull, Academic Press, Orlando, 1984)). In temperature-responsive plants, exposure to cold temperatures promotes flowering by a process known as vernalization. Vernalization affects meristems directly, perhaps by causing them to become competent to perceive flowering signals

(Lang, Encyclopedia of Plant Physiology, 15(Part 1):1371-1536, (ed., W. Ruhland, Springer-Verlag, Berlin, 1965)). Other environmental cues that can affect flowering include light quality and nutritional status.

[0006] The developmental state of the plant can also influence flowering time. Most species go through a juvenile phase during which flowering is suppressed and then undergo a transition to an adult phase in which the plant becomes competent to flower (Poethig, Science, 250:923-930 (1990)). This "phase change" permits the plant to reach a proper size for productive flowering.

[0007] The influence of the development state of a plant on flowering timing is controlled along developmental flowering pathways. In the flowering literature, the developmental flowering pathways are often referred to as autonomous to indicate that they do not involve the sensing of environmental variables. However, it is unlikely that the autonomous and environmental pathways are entirely distinct. For example, day-neutral species of tobacco typically flower after producing a specific number of nodes and, thus, could be considered as flowering entirely through an autonomous pathway. However, grafting studies have indicated that both day-neutral and photoperiod-responsive tobacco species respond to similar translocatable flowering signals (Lang et al., Proc. Natl. Acad. Sci., USA, 74:2412-2416 (1977); McDaniel et al., Plant J., 9:55-61 (1996)). Accordingly, aspects of the underlying biochemistry of these pathways appear to be conserved.

[0008] Genetic analyses of several species has identified genes that affect the time in which a plant begins to flower. The most extensive genetic analysis of these genes has been performed in the plant species, *Arabidopsis thaliana.*

[0009] In *Arabidopsis*, genes which control flowering timing have been identified by two approaches. One approach has been to induce mutations in early-flowering varieties so as to elicit either late-flowering or early-flowering. Late-flowering mutations identify genes whose wild-type role is to promote flowering, while early-flowering mutations identify genes that inhibit flowering. Studies in Arabidopsis have identified over 20 loci whose mutations specifically affect flowering time, and several other loci that affect flowering time as well as other aspects of development (e.g., *det2, cop1, ga1* and *phyB*) (Koornneef et al., Ann. Rev. Plant Physiol., Plant Mol. Biol., 49:345-370 (1998); Weigel, Ann. Rev. Genetics, 29:19-39 (1995)).

[0010] A second approach to identifying flowering timing genes is to determine the genetic basis

for the naturally occurring variations in flowering time. Although early-flowering *Arabidopsis* varieties are the most commonly used varieties in the lab, most *Arabidopsis* varieties are actually late-flowering. Late-flowering varieties differ from early-flowering varieties in that the late-flowering varieties contain dominant alelles at two loci, *FRIGIDA (FRI)* and *FLOWERING LOCUS C (FLC),* which suppress flowering (Sanda et al., Plant Physiol., 111:641-645 (1996); Lee et al., Plant Journal, 6:903-909 (1994); Clarke et al., Mol. Gen. Genet., 242:81-89 (1994); Koornneef et al., Plant Journal, 6:911-919 (1994)).

[0011] Physiological analyses of the flowering timing mutants and the naturally occurring flowering timing variations indicate that flowering is controlled in *Arabidopsis* by multiple pathways (Koornneef et al., Ann. Rev. Plant Physiol., Plant Mol. Biol., 49:345-370 (1998)). For example, plants containing one group of late-flowering mutants (*fca, fpa, fve, fy, ld*) and plants containing the late-flowering *FLC* and *FRI* alleles are delayed in flowering during inductive (long-day) conditions and more severely delayed during short-day conditions. Studies have shown that vernalization of these late-flowering lines can suppress the late-flowering phenotype. Another group of late-flowering mutants (*co, fd, fe, fha, ft, fwa, gi*) exhibit minimal or no difference in flowering time when grown in short days compared to long days. This group also shows little or no response to vernalization. Moreover, double mutants within a group do not flower later than either single-mutant parent, whereas double mutants containing a mutation in each group flower later than the single-mutant parents (Koornneef et al., Genetics, 148:885-92 (1998)). A separate autonomous pathway appears to control the age or, more specifically, the developmental stage at which plants are competent to flower. This pathway is referred to as autonomous because mutations in this pathway do not affect the plant's photoperiod response. Recent studies of these mutations have shown changes in these mutants, such as alterations of trichome patterns, which indicate that such mutant plants are delayed in transitioning from the juvenile to adult states (Telfer et al., Development, 124:645-654 (1997)). Accordingly, there appears to exist parallel flowering pathways which mediate flowering time in response to environmental and developmental cues.

[0012] The time in which plants flower is of great importance in both agricultural and horticultural crops. In horticultural crops, the product is often the flower, while in food, feed or fiber crops, the product is often the flower and/or the products of flowering (i.e., fruits, seeds, or

seedpods). Understanding the molecular aspects of flowering timing control in these crops will lead to strategies for optimizing flower, fruit, and seed production. For example, accelerating the onset of flowering in certain crops may permit those crops to be grown in regions where the growing season is otherwise too short, or permit multiple crops to be grown in regions where only one crop is currently possible. In addition, preventing or substantially delaying flowering will increase the yield of the useful parts of certain crops. For example, delaying or preventing flowering in forage crops (e.g., alfalfa and clover) and vegetables crops (e.g., cabbage and related Brassicas, spinach, and lettuce) should increase crop yields. Likewise, the yields of crops in which underground parts are used (e.g., sugar beets or potatoes), may also be increased by delaying or preventing flowering. In sugar beets, the prevention of flowering will also permit more energy to be devoted to sugar production. Likewise the yield of wood and biomass crops may also be increased by delaying flowering.

SUMMARY OF THE INVENTION

[0013] The present invention is summarized in that a novel FPA protein coding sequence has been isolated and used to affect the flowering time of plants by altering the level of FPA protein activity in the cells of the plant.

[0014] The present invention includes a plant comprising in its genome a transgene encoding an FPA polynucleotide sequence, wherein the transgene alters the timing of the plants flowering as compared to non-transgenic plants of the same species. The plant may be genetically modified by the introduction of the FPA polynucleotide sequence in either the sense or antisense orientation.

[0015] The present invention also includes a genetic construct comprising an FPA polynucleotide sequence and a promoter that promotes expression of the sequence in plants. The present invention is also directed towards polynucleotide sequences representing genes that function in regulating FPA protein activity, and which, when expressed, alter the flowering time of the plant in which it is introduced.

[0016] The present invention also includes a method for altering the flowering time of a plant using the FPA polynucleotide sequence described above, and a method for down-regulating FLC mRNA activity using the FPA polynucleotide sequence described above.

[0017] The present invention is also a seed, comprising in its genome a genetic construct comprising an FPA polynucleotide sequence and a promoter that promotes gene expression in plants.

[0018] The present invention is also a plant cell comprising in its genome a genetic construct comprising an FPA polynucleotide sequence and a promoter capable of promoting gene expression in plants.

[0019] It is an object of the present invention to provide a method and a tool for altering the flowering timing in plant species. The flowering timing can be made earlier or later by affecting the level of the FPA protein in such plants.

[0020] Other objects advantages and features of the present invention will become apparent from the following specification.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] None.

DETAILED DESCRIPTION

[0022] The present invention is directed at the nucleotide and protein sequences for the FPA gene in plants. The FPA gene has been discovered to be a gene which acts to promote floral induction in plants during both long and short day photoperiods. It is disclosed here that the absence or suppression of FPA activity in plants results in a delay of floral induction in both long and short day photoperiods, while the added expression of FPA in plants causes an earlier flowering timing relative to non-transgenic plants of the same species.

[0023] The lifetime of a plant can be divided into at least two phases referred to as the vegetative phase and the reproductive phase. During the vegetative phase, most commercially important crop plants grow continuously, increasing in both size and leaf number, until the reproductive phase is reached. The reproductive phase begins with flowering initiation. At that point much of the plant's further growth is directed towards the growth (or development) of flowers, fruits, and seeds utilized in reproduction.

[0024] We have discovered that the *Arabidopsis* FPA gene is capable of altering floral induction in a wide range of genetic and physiologic conditions such that it is anticipated that the FPA gene

may be a useful tool for regulating the flowering time in many plant species. For example, the FPA gene can accelerate flowering in several late-flowering mutant backgrounds (*e.g., fve-2* and *fca-1*), and can fully compensate for the addition of two naturally occurring genes, FRI and FLC, which confer late-flowering phenotypes. Because FRI and FLC may be responsible for regulating the flowering time in plant species other than *Arabidopsis*, it is anticipated that the FPA gene may also be capable of altering the flowering time of such species as well. Our studies have also shown that the overexpression of FPA can compensate for the delaying effect caused by short days on floral induction. Accordingly, the added expression of FPA may be used to alter the flowering time in species having a photoperiod requirement for flower initiation. In addition, our studies have shown that the overexpression of FPA has the additional effect of decreasing FLC mRNA in plants containing the FLC gene, suggesting that FPA may be a useful tool for down-regulating FLC activity.

[0025] The present invention provides a method for altering the time of flower initiation in a plant by introducing into the genome of the plant an FPA polynucleotide sequence capable of up-regulating or down-regulating FPA activity in the cells of the plant. Upon introduction into the genome of a plant, the FPA polynucleotide sequence can act to augment the activity of an endogenous gene regulating the time in which the plant would typically initiate flowering. For instance, a second copy of the FPA gene can be introduced into a plant to increase the amount of FPA activity present in the plant and cause early-flowering. Expression of a portion of a polypeptide encoded by the FPA gene can also lead to a delay of flowering in a plant. This polypeptide portion which leads to the delay of flowering in a plant can be referred to as a dominant negative mutant. Fragments of the FPA gene can also act to decrease activity of an endogenous FPA gene by modifying the expression of the endogenous FPA gene. For instance, expression of the complement of the FPA gene can result in an antisense RNA fragment which will suppress FPA activity and lead to delay the activation of the flowering time in the plant. In addition, the expression of an FPA polynucleotide sequence of the present invention may also result in a delay of flowering by cosuppression. Another method to alter the activity of the FPA gene is to introduce into a plant's genome a fragment that encodes an antibody or other polypeptide that would bind to the FPA gene, or its RNA, or a protein encoded by the FPA gene and render it inactive or less active.

[0026] The present invention also provides a method for altering FLC mRNA activity in plants containing the FLC gene by introducing into the genome of the plant an FPA polynucleotide sequence capable of suppressing FLC mRNA activity. Upon introduction into the genome of a plant, the FPA polynucleotide sequence may either increase the amount of FPA activity present in the plant to cause a decrease in FLC mRNA activity, or cause the expression of a portion of a polypeptide or nucleotide sequence which leads to a decrease in FLC mRNA activity. In general, FLC mRNA is upregulated by the presence of the FRIGIDA (FRI) gene. In the genetic background in plants containing both FRI and FLC, FLC represses flowering based on the quantity of the FLC message. Accordingly, overexpression of FPA in a FRI/FLC containing plant results in the removal of FLC message and concurrent early flowering.

[0027] The identification and characterization of the FPA gene from *Arabidopsis thaliana* is described in the examples below. While this gene has existed previously in its native, or altered, state in plants, this disclosure is believed to be the first disclosure of the FPA gene in its isolated form. By isolated form, it is meant that the genes have been isolated from their host plants. The sequence for the entire FPA gene is set forth in SEQ ID NO:1. The cDNA protein coding sequence for the FPA gene is set forth in SEQ ID NO:2, and its deduced amino acid sequence is set forth in SEQ ID NO:3. An analysis of this data indicates that the FPA gene shares some sequence homology with other known RNA binding proteins. Because of the high degree of sequence homology across plant species, it is anticipated that the present invention is capable of use in a large number of plants. In addition, it is anticipated that the FPA polynucleotide sequence of the present invention may also be used to isolate other FPA genes in other plant species in order to obtain other FPA polynucleotide sequences having similar effects.

[0028] As used herein, "FPA" refers to the *Arabidopsis* FPA gene (SEQ ID NO:1), as well as the FPA cDNA (SEQ ID NO:2) and the analogous gene sequences from other plants, and the variations and mutants thereof which retain flowering functionality. It is expected that most flowering plants contain FPA genes homologous to the *Arabidopsis* FPA gene. Given the high degree of sequence homology across plant species, it is reasonable to expect that FPA genes from any plant, of which the *Arabidopsis* FPA gene is but one example, could be used in the practice of the present invention. For example, FPA genes from plants that are raised for their agricultural or horticultural value may be used in the practice of the present invention.

[0029] It is specifically contemplated that any FPA polynucleotide sequence could be used in the practice of the present invention. "FPA polynucleotide sequence" is defined to include any plant DNA sequence which expresses an FPA gene, or which is capable of overexpressing or reducing the expression of the FPA gene native to the plant in which the FPA polynucleotide sequence is introduced. An FPA polynucleotide sequence may be an unmodified sequence (such as SEQ ID NO:1) isolated from any plant, a cDNA sequence (such as SEQ ID NO:2) derived from any plant, a genomic or cDNA sequence that is modified to contain minor nucleotide additions, deletions, or substitution, or a synthetic DNA sequence. The term is also intended to apply to analogous sequences from other plants as well as allelic variations and mutations which are still capable of controlling FPA activity.

[0030] Analogous sequences (homologs) include genes from *Arabidopsis* and other plant species having a certain percentage of identity with SEQ ID NO:2. Identity is a relatedness that can be determined by, but not limited to, nucleic acid hybridization techniques, computer searches of databases, computer or manual comparisons of amino acid and nucleotide sequences, and protein detection with the use of FPA-specific antibodies. Two analogous nucleotide sequences are "similar" if they can be aligned so that a percentage of corresponding residues are identical. For example, two nucleotide sequences are analogous if the have greater than about 31%, more preferably at least about 50%, even more preferably at least about 70%, and most preferably at least about 80% identity to each other.

[0031] Homologs also include polypeptides from *Arabidopsis* and other species having a certain level of identity with the polypeptide encoded by SEQ ID NO:1 or SEQ ID NO:2. Homologs also include coding regions and polypeptides that function comparable to an FPA coding region on an FPA polypeptide. For example, a polypeptide homolog includes, without limitation, a polypeptide having greater than about 31%, more preferably at least about 50%, even more preferably at least about 70%, and most preferably at least about 80% identity to the RNA binding region of the FPA protein. Whether a coding region or a polypeptide is an homolog can be determined by expressing the coding region and/or the polypeptide in *Arabidopsis* and evaluating the effect on flower initiation. Described in the examples below are tests to determine whether a particular FPA gene does, in fact, act to delay or promote flowering. By testing these

genes, using *Arabidopsis* as a model, the activity of a particular FPA gene can be confirmed.

[0032] FPA polynucleotide sequence is also intended to include fragments of an FPA gene which are capable of altering the time of flower initiation in a plant. Such fragments include polymeric forms of nucleotides of any length, either ribonucleotides or deoxynucleotides, and includes both double- and single-stranded DNA (both genomic and cDNA) and both double- and single-stranded RNA. A fragment may also include both coding and non-coding regions that can be obtained directly from a natural source (e.g., a plant), or can be prepared with the aid of recombinant or synthetic techniques. Fragments also include polynucleotide sequences, wherein the complement of the polynucleotide sequence hybridizes to SEQ ID NO:1 or SEQ ID NO:2 under standard hybridization conditions. During hybridization the entire nucleotide sequence of the complement can hybridize with SEQ ID NO:1 or SEQ ID NO:2, or a portion thereof. Preferably, at least about 20 nucleotides of the complement hybridize with SEQ ID NO:1 or SEQ ID NO:2, more preferably at least about 50 nucleotides, and most preferably at least about 100 nucleotides. In the preferred embodiment, such fragments include those encoding a polypeptide which is involved in altering the timing of flower initiation in a plant. Portions of such a polypeptide and homologs of such a polypeptide are also included in the present invention, provided they have the ability to alter the time of flower initiation.

[0033] By "transgene" it is meant to describe an artificial genetic construction carried in the genome of a plant and inserted in the plant or its ancestor by gene transfer. Such transgenes are transmissible by normal Mendelian inheritance once inserted. By "transgenic plant" it is meant any plant modified by the introduction of a transgene into the genome of one or more plant cells, which can generate whole, sexually competent, viable plants.

[0034] As used herein, the term "isolated" means that a polypeptide or polynucleotide fragment is either removed from its natural environment or synthetically derived. Preferably, the polypeptide or polynucleotide is purified, i.e., essentially free from any other polypeptides or polynucleotides, respectively, and associated cellular products or other impurities.

[0035] "Polypeptide" as used herein refers to a polymer of amino acids and does not refer to a specific length of a polymer of amino acids. Thus, for example, the terms peptide, oligopeptide, protein, and enzyme are included within the definition of polypeptide. This term also includes

post-expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations, and the like. "Percentage amino acid identity" refers to a comparison of the amino acids of two polypeptides as described herein.

[0036] "Flower initiation" refers to the transition of a shoot meristem to the formation of flower primordia. Flower initiation can be determined by microscopic analysis to determine the formation of flower primordia, or by the naked eye. The "leaves" of a plant present at flower initiation include all the leaves present on a plant.

[0037] A "coding region" is a linear form of nucleotides that encodes a polypeptide, usually via mRNA, when placed under the control of appropriate regulatory sequences. The complement of the coding region can encode an antisense RNA polynucleotide fragment when placed under the control of appropriate regulatory sequences. The boundaries of a coding region are generally determined by a translation start codon at its 5' end and a translation stop codon at its 3' end. "Expression" of a coding region refers to those processes that are required to result in a polypeptide, including, for instance, transcription of the coding region and translation of the mRNA encoded by the coding region.

[0038] The present invention provides for a transgenic plant having in its genome a transgene containing a sense or antisense FPA polynucleotide sequence which causes the plant to have an altered flowering time as compared to non-transgenic plants of the same species. The FPA polynucleotide sequence may include, without limitation, sequences which encode polypeptides involved in the promotion of flowering, or produce antisense RNA, or are part of a construct involved in cosuppression, or encode an antibody or other binding polypeptides that inactivate or reduce FPA activity. Also provided are plant cells and plant tissues derived from the transgenic plant of the present invention, and seeds which can germinate into a transgenic plant described herein.

[0039] Plants included in the invention are any flowering plants amenable to transformation techniques, including both monocotyledonous and dicotyledonous plants. Examples of monocotyledonous plants include, but are not limited to, vegetables such as asparagus, onions and garlic; cereals such as maize, barley, wheat, rice, sorghum, pearl millet, rye and oats; and grasses such as forage grasses and turfgrasses. Examples of dicotyledonous plants include, but are not limited to, vegetables, feed, and oil crops such as tomato, beans, soybeans, peppers,

lettuce, peas, alfalfa, clover, Brassica species (e.g., cabbage, broccoli, cauliflower, brussel sprouts, rapeseed, and radish), carrot, beets, eggplant, spinach, cucumber, squash, melons, cantaloupe, sunflowers; fiber crops such as cotton; and various ornamentals such as flowers and shrubs.

[0040] It is specifically envisioned that transgenic plants according to the present invention can be made with a transgene for an FPA polynucleotide sequence which selectively down-regulates or up-regulates FPA activity. Several techniques are known in the art for either down-regulating or up-regulating the activity of such endogenous plant genes. For example, extra copies of the FPA gene (SEQ ID NO:1) or its cDNA (SEQ ID NO:2) can be introduced into a plant to up-regulate FPA activity. On the other hand, a polynucleotide fragment encoding an FPA antisense RNA (i.e., SEQ ID NO:4) or cosuppression construct can be used to down-regulate FPA activity. A construct producing an antisense RNA would generally include a promoter driving the production of an antisense RNA polynucleotide molecule complementary to an mRNA produced by the FPA gene. The antisense RNA polynucleotide can be a portion of the corresponding FPA mRNA, as it has been demonstrated that such portions can function effectively to suppress gene expression (Bariola et al., *Plant Physiol.*, **119**, 331-342 (1999); Kang et al., *Plant Mol. Biol.*, **38**, 1021-1029, 1998).

[0041] Another down-regulating method is to use cosuppression. Cosuppression is a poorly understood phenomenon by which insertion of an artificial gene construct into a plant occasionally causes suppression of both the inserted gene and any other gene homologous to it. In general, a cosuppression construct will raise the level of FPA mRNA, or a fragment of the mRNA, to a level that the cell will decrease expression of both the endogenous FPA gene and transgene (Kasschau et al., *Cell*, **95**, 461-470, 1998). Cosuppression can occur by introducing an FPA polynucleotide fragment that includes an FPA coding region (i.e., SEQ ID NO:6) , or portion thereof (i.e., SEQ ID NO:5), which is identical to the endogenous FPA coding region.

[0042] Another method to modify FPA activity is to introduce into a plant's genome a polynucleotide fragment that encodes an antibody or other polypeptide that would bind to the FPA polypeptide and render it inactive or less active.

[0043] Another method to modify FPA activity is to introduce into a plant's genome polynucleotide fragments encoding dominant-negative versions of a flower time regulation

polypeptide. Dominant-negative mutants are proteins that actively interfere with the function of normal, endogenous proteins. Thus, the action of a gene can be blocked without inactivating the structural gene itself or its RNA.

[0044] Transgenic plants of the invention are produced by contacting a plant cell with a genetic construction, for example, that includes a FPA polynucleotide sequence as described above. To be effective once introduced into plant cells, the FPA polynucleotide sequence is typically operably associated with a promoter capable of causing transcription and expression of a the polypeptide or mRNA encoded by the FPA polynucleotide sequence. A polyadenylation sequence or transcription control sequence, also recognized in plant cells, may also be employed. It is preferred that the genetic construction harboring the FPA polynucleotide sequence also contain one or more selectable marker genes so that the transformed cells can be selected from nontransformed cells in culture.

[0045] The transformation of plants in accordance with the invention may be carried out in essentially any of the various ways known to those skilled in the art of plant molecular biology. As used herein, the term "transformation" means alteration of the genotype of a host plant by the introduction of an FPA polynucleotide fragment of the present invention.

[0046] For example, an FPA polynucleotide fragment can be introduced into a plant cell utilizing *Agrobacterium tumefaciens* mediated transformation. This method of transformation requires that the FPA polynucleotide sequence be incorporated into the transferred DNA region (T-DNA) of a plasmid that can replicate in Agrobacterium. Methods involving the use of Agrobacterium include, but are not limited to: 1) co-cultivation of Agrobacterium with cultured protoplasts; 2) transformation of plant cells or tissues with Agrobacterium; or 3) transformation of seeds or shoots by infiltration of a suspension of Agrobacterium cells as described by (Bechtold et al., *C.R. Acad. Sci. Paris*, **316**, 1194, 1993) and exemplified in the Examples herein.

[0047] Alternatively, a FPA polynucleotide sequence can be introduced into a plant cell by contacting the plant cell using mechanical, electrical, or chemical means. For example, the sequence can be mechanically transferred by microinjection directly into plant cells by use of micropipettes. Alternatively, the sequence may be transferred into the plant cell by using polyethylene glycol which forms a precipitation complex with genetic material that is taken up by the cell. The FPA polynucleotide sequence can also be introduced into plant cells by

electroporation (Fromm et al., *Proc. Natl. Acad Sci., U.S.A.*, **82**, 5824, 1985). In this technique, plant protoplasts are electroporated in the presence of vectors or nucleic acids containing the relevant polynucleotide fragments. Electrical impulses of high field strength reversibly permeabilize membranes allowing the introduction of nucleic acid molecules. Electroporated plant protoplasts reform the cell wall, divide and form a plant callus. Selection of the transformed plant cells with the transformed FPA polynucleotide sequences can be accomplished using phenotypic markers as are well known in the art.

[0048] Another method for introducing the FPA polynucleotide sequences of the present invention into a plant cell is by high velocity ballistic penetration using small particles with the sequences to be introduced contained either within the matrix of small beads or particles, or on the surface thereof (Klein et al., *Nature*, **327**, 70, 1987).

[0049] Viruses such as the Cauliflower mosaic virus (CaMV) may also be used as a vector for introducing the FPA polynucleotide sequences into plant cells (U.S. Pat. No. 4,407,956). The CaMV viral DNA genome is inserted into a parent bacterial plasmid creating a recombinant DNA molecule which can be propagated in bacteria. After cloning, the recombinant plasmid again may be cloned and further modified by introduction of the desired polynucleotide sequence. The modified viral portion of the recombinant plasmid is then excised from the parent bacterial plasmid, and used to inoculate the plant cells or plants.

[0050] The experience to date in the technology of plant genetic engineering is that the method of gene introduction is not of particular importance in the phenotype achieved in the transgenic plant. Once the plant has been genetically engineered, and a transgenic plant has been created, the method of transformation of the original plant becomes irrelevant. A transgene inserted into the genome of one plant is then fully inheritable by progeny plants of the original genetically engineered plant by normal rules of classical plant breeding.

[0051] To make a transgenic plant, as is known to those of skill in the art, one needs to make a genetic construction capable of expressing the inserted protein coding sequence, whether foreign or endogenous, in the plant to which it has been introduced. The tools and techniques for making genetic constructions that will express proteins in plants are widely known in the art of plant genetics. In general, such genetic constructions include a polynucleotide sequence (e.g., the

coding region, or a portion thereof, of the FPA gene) operably associated with a promoter capable of promoting expression of the polynucleotide sequence in the plant.

[0052] The promoter used in the genetic construction of the present invention can be either a constitutive promoter or an inducible promoter. Examples of constitutive promoters useful in plant genetic constructions include, without limitation, the 35S RNA and 19S RNA promoters of the cauliflower mosaic virus (Brisson et al., *Nature*, **310**, 511, 1984); the opine synthase promoters carried on the tumor-inducing plasmids of Agrobacterium tumefaciens such as the nopaline synthase promoter (Ebert et al., *PNAS,* **84**, 5745, 1987) and the mannopine synthase promoter (Velten et al., *EMBO J.* **3**, 2723 1984). Tissue-specific promoters may also be used in the present invention. An example of a tissue-specific promoter is the H4A748 promoter expressed in shoot meristems (Atanassova et al., *Plant J.,* **2**, 291, 1992).

[0053] An inducible promoter suitable for use in the present invention should: 1) provide undetectable or minimal expression in the absence of an inducer; 2) provide sufficiently high expression in the presence of an inducer to produce the desired modification of flowering time; and 3) induce expression of the desired polypeptide or mRNA without substantially altering the physiology of the plant material (other than the effects of the polypeptide itself). Examples of inducible promoters suitable for use in the present invention include, but are not limited to, heat shock promoters such as soybean hsp17.5E or hsp17.3 (Gurley et al., *Mol. Cell Biol.* **6**, 559, 1986), light-regulated promoters such as the promoter for the small subunit or ribulose bisphosphate carboxylase (ssRUBISCO) (Coruzzi et al., *EMBO J.* **3**, 1671, 1984; Broglie et al., *Science* **224**, 838, 1984), chemical-regulated promoters such as Maize In2-1 and 2-2 which are regulated by benzenesulfonamides e.g., herbicide safeners (Hershey et al., *Plant Mol. Biol.*, **17**, 679, 1991) and alcA and alcR promoter/transcription factor system that is induced by the application of ethanol (Caddick et al., *Nat. Biotech.*, **16**, 177, 1998). Other promoters of gene expression will be known to those skilled in the art.

[0054] The promoter utilized should be capable of producing sufficient expression of the FPA protein or a portion thereof, or RNA (including part of the RNA sequence or the RNA sequence in entirety to cause cosuppression or an antisense RNA expression) to modify the flowering time of the plant. The promoters used in the constructs of the present invention may be altered, if desired, to modify their expression characteristics.

[0055] Optionally, a selectable marker may be associated with the FPA polynucleotide sequence. As used herein, the term "marker" refers to a gene encoding a trait or a phenotype which permits the selection of, or the screening for, a plant or plant cell containing the marker. Preferably, the marker gene is an antibiotic resistance gene whereby the appropriate antibiotic can be used to select for transformed cells from among cells that are not transformed. Examples of suitable selectable markers include adenosine deaminase, dihydrofolate reductase, hygromycin-B-phosphotransferase, thymidine kinase, xanthine-guanine phospho-ribosyltransferase and amino-glycoside 3'-O-phosphotransferase II (which confers kanamycin, neomycin and G418 resistance). Other suitable markers will be known to those of skill in the art.

[0056] Transgenic plants according to the present invention may exhibit early or late flowering initiation dependent upon the transgene introduced into the plant. For example, flowering initiation (on average) in a transgenic plant having a transgene that down-regulates FPA activity may occur at least about 3 days, or at least about 7 days, or at least about 12 days, or at least 30 days, or at least about 60 days after initiation of flowering in the same plant without the transgene. Alternatively, flowering initiation (on average) in a transgenic plant having a transgene that up-regulates FPA activity may occur at least about 3 days, or at least about 7 days, or at least about 12 days, or at least about 30 days, or at least about 60 days before initiation of flowering in the same plant without the transgene.

[0057] The difference in the length of time to the onset of the flowering stage of a transgenic plant relative to a non-transgenic plant can also be measured by determining the difference in the number of leaves at the time of flower initiation on the transgenic plant as compared to a non-transgenic plant of the same species. Preferably, the transgenic plant having a transgene which down-regulates FPA activity exhibits at least about 50% more, preferably at least about 100% more, more preferably at least about 400% more, and most preferably at least about 800% more leaves at flower initiation than the non-transgenic plant. Alternatively, the transgenic plant having a transgene which up-regulates FPA activity exhibits at least about 10% fewer, preferably at least about 50% fewer, and most preferably at least about 80% fewer leaves at flower initiation than the same non-transgenic plant.

[0058] Isolated FPA polynucleotide sequences of the invention can be obtained by several methods. For example, they can be isolated using procedures which are well known in the art.

These include, but are not limited to: 1) hybridization of detectably labeled probes representing all or part of the *Arabidopsis* FPA gene to genomic or cDNA libraries to detect similar nucleic acid sequences; 2) antibody screening of expression libraries to detect similar structural features; 3) synthesis by the polymerase chain reaction (PCR); and 4) chemical synthesis of a nucleic acid molecule. Sequences for specific coding regions of genes can also be found in GenBank, the National Institutes of Health computer database. The coding region can then be isolated and ligated into a vector as is well known in the art. Probes useful in the invention include those made using the entire FPA coding region or portions thereof.

[0059] FPA belongs to a class of genes known as RNA-binding proteins. These genes and the polypeptides they encode typically have several conserved domains. The RNP2/RNP1 RNA binding regions contain similarity to other members of the family of RNA-binding proteins. Therefore, probes that contain these regions may be useful in the isolation of FPA-homologous sequences. Additionally, RNA-binding proteins also contain other domains useful for identification of homologues (such as an acidic C-terminus). Probes to these less conserved regions may also be used to isolate FPA homologues.

[0060] In a preferred embodiment, the invention includes a method of producing a genetically modified plant characterized as having modified timing of flowering, said method comprising constructing a genetic construct as described above; introducing the construct into a plant cell; growing a plant from said transformed plant cell and selecting a plant that has received the genetic construct; and growing the plant under conditions that allow expression of the FPA polynucleotide sequence to alter the flowering time of the plant. As used herein, the term "introducing" refers to any means of introducing the transgene into the plant cell, including chemical and physical means as described above.

[0061] Normally, a transformed plant cell is regenerated to obtain a whole plant from the transformation process. The term "growing" or "regeneration" as used herein means growing a plant from a protoplast, a plant cell, a group of plant cells, a plant part (including seeds), or a plant piece (e.g., from a protoplast, callus, or tissue part).

[0062] In vegetatively propagated crops, the mature transgenic plants are propagated by the taking of cuttings or by tissue culture techniques to produce multiple identical plants. Selection

of desirable transformed plants is made and new varieties are obtained and propagated vegetatively for commercial use.

[0063] In seed-propagated crops, the mature transgenic plants can be self crossed to produce a homozygous inbred plant. The inbred plant produces seed containing the newly introduced polynucleotide fragment, preferably, heterologous polynucleotide fragment. These seeds can be grown to produce plants that would produce the selected phenotype, modified timing of flowering.

[0064] Parts obtained from the regenerated plant, such as flowers, seeds, leaves, branches, fruit, and the like are included in the invention, provided that these parts comprise cells that have been transformed as described. Progeny and variants, and mutants of the regenerated plants are also included within the scope of the invention, provided that these parts comprise the introduced polynucleotide fragments.

[0065] Plants exhibiting modified timing of flowering can be selected by visual observation. Commercially important crop plants have been bred for desirable characteristics, including uniformity in the time the plants are ready for harvesting. This has resulted in a high degree of uniformity in the number of leaves present on each plant in a population of plants grown under the same conditions. Due to the uniformity in the number of leaves present, alterations in the time of flower initiation can often be measured as a function of the number of leaves on a plant. For instance, if flower initiation is activated early in a plant, that plant will have fewer leaves relative to the same type of (or unmodified) plant grown under the same conditions that does not activate flower initiation early. Moreover, a plant that activates flower initiation early can also be said to have a shortened vegetative phase relative to the same type of plant grown under the same conditions that does not activate flower initiation early. Likewise, if flower initiation is repressed such that the plant undergoes flower initiation later, that plant will have more leaves relative to the same type of plant grown under the same conditions that does not repress flower initiation until later. Moreover, a plant that represses flower initiation may also be said to have a prolonged vegetative phase relative to the same type of plant grown under the same conditions that does not repress flower initiation. Alterations in the time of flower initiation can also be measured as a function of time.

[0066] The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only and are not intended to limit the scope of the invention.

[0067] While the examples set forth below are executed in *Arabidopsis*, due to the simplicity in the genetic manipulation of that plant, the same techniques will work in other plants species. In fact, the high degree of sequence identity across plant species suggests that an FPA gene from one plant species will function, as a general rule, in other plants species.

EXAMPLES

Isolation of the FPA Gene.

[0068] The FPA gene was isolated by positional cloning performed on *fpa* mutants of the Columbia ecotype of *Arabidopsis*. Unlike the Columbia ecotype, which flowers after producing 10-12 vegetative leaves, mutants which have lost FPA function flowered after producing 80-90 vegetative leaves.

[0069] Loss of function *fpa* alleles were created by both ethylmethanesulfonate (EMS: *fpa-1*, *fpa-2*, and *fpa-4*), and by Agrobacterium-mediated transfer DNA (T-DNA) mutagenesis (*fpa-3*, *fpa-5*, and *fpa-6*). The *fpa-4* allele was determined to be a deletion of a 35kb sequence which eliminated FPA function. This deletion was contained in the BAC 1O24 and sequenced by TIGR (http://www.tigr.org/tdb/at/atgenome/completed.html).

[0070] BAC 1O24 was then used to generate a library of small clones containing 5-15 kb of DNA. These small clones were then transformed into *fpa-3* via Agrobacterium-mediated transformation to determine the region in the plant genome that contained the FPA gene.

[0071] Several independent transformants were observed to rescue the *fpa* mutant phenotype (Table 1). The rescuing clones were then sequenced and it was discovered that all of the rescuing clones contained the same region of DNA. Analysis of this data further indicated that FPA was an RNA-binding protein, as indicated in SEQ ID No. 3.

Table 1

Arabidopsis line	Rosette leaf number at flowering
Columbia (wild-type)	10-12
fpa mutant (Columbia)	80-90
fpa-3 (WS)	50-65
Wild-Type (WS)	7-8
fpa-3 + rescue clone 1	6-7
fpa-3 + rescue clone 2	7-8
fpa-3 + rescue clone 4	7-8

[0072] Additional *fpa* alleles were then analyzed. One allele (*fpa-3*) was found to contain a deletion that removed 2.5 kb of a promoter and 5' FPA coding region. The other two (*fpa-5*, and *fpa-6*) alleles were found to contain T-DNA insertions in the *FPA* coding region. Two other independent *fpa* alleles (*fpa-1* and *fpa-2*) which were generated by EMS mutagenesis were also sequenced. Both of these alleles contained DNA base changes in the RNA-binding protein that resulted in stop codons which truncated the FPA protein.

Overexpression of *FPA* results in Altered Floral Induction

[0073] Expression using several different constructs containing partial or full-length *FPA* sequences yielded plants that had altered flowering times compared to that of non-transformed control plants. *FPA* has been used to generate both early and late-flowering phenotypes in transgenic Arabidopsis. This data implies that *FPA* is a potent regulator of floral induction and can either compensate for and/or bypass other blocks to floral induction. Due to the ability of *FPA* to produce altered flowering in many different genetic backgrounds and physiologic growth conditions it is anticipated that *FPA* will be able to regulate flowering in species other than Arabidopsis.

Early Flowering in Short Days due to Expression of the Full-Length *FPA* Gene

[0074] The wild-type summer-annual WS accession of Arabidopsis was transformed with constructs containing the entire FPA gene using Agrobacterium-mediated transformation. The construct was prepared using the isolated *FPA* gene (amplified from genomic Arabidopsis DNA using the primers ATGGCGTTATCTATGAAGCCATTCAGAGCC and TCAAGGCCCCTGTCCAGCCGGAGTACC), and a 35S CaMV promoter. Successful transformants were then collected and grown in conditions to allow assessment of the FPA overexpression on flowering time.

[0075] To assay for altered flowering time, transformants were grown in both long day, and short day conditions. Overexpression caused early flowering in short day conditions (Table 2), as typical wild-type Arabidopsis strains generally flower after producing 25-30 leaves in short days.

Table 2

Arabidopsis line	Rosette leaf number at time of flowering
WS (wild type) in Long days	7-8
WS + *35S::FPA* in Long days	6-9
WS (wild type) in Short days	25-30
WS + *35S::FPA* in Short days	7-8* and 25-30*

* Two types of plants were observed in SD: early flowering and wild-type flowering. Approximately %40 were of the early-flowering phenotype.

Generation of Early-Flowering Plants from Late-Flowering Genetic Backgrounds by Overexpression of *FPA*

[0076] The *FPA* overexpression construct described in the generation of early-flowering plants in short days was used to investigate the ability of *FPA* to promote flowering in several genetic backgrounds that are later flowering than the WS accession. The effects of overexpression of *FPA* was determined in the following genetic backgrounds: *fve-2* mutant, *fca-1* mutant, and the naturally occurring dominant *FRIGIDA (FRI)/FLC* gene pair background. The results verify that *FPA* reduces the flowering time in the late-flowering plants to that of their respective wild-type plants (Table 3).

Table 3

Genetic Background	Rosette leaf number at time of flowering in Long Days
L*er* (wild-type)	7-8
L*er fve-2*	18-22
L*er fve-2 + 35S::FPA*	7-8
L*er fca-1*	20-24
L*er fca-1 + 35S::FPA*	7-8
Columbia (wild-type)	10-12
Columbia + *FRI/FLC*	70-90
Columbia + *FRI/FLC + 35S::FPA*	10-12

The Generation of Late-Flowering Arabidopsis using *FPA*

[0077] Using the *FPA* polynucleotide sequence (SEQ ID NO:2), late-flowering Arabidopsis were generated using both antisense and cosuppression transgenes. An antisense construct was generated using the 5' coding region of *FPA* (amplified by the primers 5' AAGACTTTAAAGGAGATGTTCAGCC and 5' CCTTTCCCATAGGTACACAACGAGC) and expressing the opposite strand under control of the CaMV 35S promoter. Primary transformants that displayed delayed flowering were selected and progeny were replanted. Upon replanting, late-flowering plants that phenocopied *fpa* mutants were isolated (i.e., flowered after producing 60 leaves).

[0078] Late-flowering Arabidopsis were likewise generated through cosuppression of *fpa* by overexpressing portions of the *FPA* polynucleotide sequence in the sense orientation with the 35S promoter. This approach also resulted in plants with delayed flowering that phenocopied *fpa* mutants (Table 4). Several constructs were able to produce late-flowering plants due to cosuppression. For example, constructs designed for overexpression of the full length *FPA* coding sequence and overexpression of partial fragments contained within the full-length *FPA* construct were able to generate late-flowering cosuppression phenotypes. Because little is known of the mechanism of cosuppression it was difficult to predict what features of a gene were advantageous for producing cossuppression. However, expression of either partial or full-length coding regions of FPA was sufficient to produce late-flowering plants through cosuppression

mechanisms.

Genetic background	Rosette leaf number at time of flowering in Long Days
Columbia (wild-type)	10-12
Columbia + FPA cosuppression construct	55-80

Overexpression of FPA decreases *FLC* mRNA

[0079] The *FLC* gene is a central floral repressor that is responsible for delaying flowering in many species. To further investigate the role of *FPA* in causing early flowering in this background, *FLC* messenger RNA was quantified by RT-PCR (reverse-transcription based polymerase chain reaction). In the control plants that did not contain overexpressed *FPA*, there was substantial *FLC* message detected (Table 5). However, in the early-flowering *35S::FPA*-containing plants there was no detectable *FLC* message, indicating that the overexpression of *FPA* in an *FLC*-expressing late-flowering background results in the removal of *FLC* message, and early flowering. (See, Table 3.) Accordingly, the introduction of *35S::FPA* into a plant expressing *FLC* message provides a system to selectively remove *FLC* message from that plant.

Table 5

Arabidopsis Genetic Background	*FLC* messenger RNA amount
Columbia + *FRI/FLC*	+++
Columbia + *FRI/FLC* + *35S::FPA*	none detectable